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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating 2017: 5.03
TPI 2017; 6(11): 393-397

www.thepharmajournal.com
Received: 24-09-2017
Accepted: 25-10-2017

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Experimental study of goat's rue (*Galega Officinalis* L.) herb and its liquid extracts

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Abstract
With an objective to develop criteria for further standardization techniques of extracts, obtained from above-ground parts of promising hypoglycemic plant goat's rue (*Galega officinalis* L.), we have carried out analysis of contents of phenolic compounds and microelements.
The article describes the results of the research concerning qualitative and quantitative composition of phenolic compounds in ethanolic extracts of the goat's rue (*Galega officinalis* L.) herb by the methods of high-performance liquid chromatography and spectrophotometry. The results of detection of phenolic compounds in aqueous-alcoholic extracts from aerial portion of goat's rue showed the occurrence of 48 compounds, amongst which 7 compounds were identified, such as caffeic acid, ferulic acid, chicoric acid, rutin, quercetin, hyperoside, and apigenin. The optimal extractant for the maximum extraction of the flavonoids' totality from goat's rue (*Galega officinalis* L) herb has been determined and the fact that the highest yield of flavonoids (0.097%) was extracted with 50% alcoholic extract has been established.
The outcomes of this experimental study carried out by the method of atomic absorption spectrometry with electrothermal atomization, for the content of trace elements, which might have an impact for the herb's hypoglycemic properties, were as follows: manganese – 23.8 µg/g, copper – 14.2 µg/g, selenium – 0.27 µg/g.

Keywords: Goat's rue, *Galega officinalis* L., hypoglycemic, flavonoids, hydroxycinnamic acids, microelements

Introduction
Diabetes mellitus affects approximately 4% of the population worldwide and is estimated to increase by 5.4% in 10 upcoming years. Medicinal plants may play an important role in the management of blood glucose through different mechanisms, e.g. due to contents of insulin-like substances, inhibition of insulin's activity or an increase of beta β-cells in the pancreas by activation of regeneration of these cells, or serving as antioxidants by reducing the oxidative stress in the pancreas [1].
Herbal remedies are gaining popularity because of several advantages such as a comparatively lower incidence of side-effects at recommended dosages, better patient tolerance, relatively low cost, and acceptance due to a long history of use [2]. Medicinal plants with sufficient natural resources, which are applied in traditional and folk medicine to treat diabetes mellitus, are objects of particular scientific interest. The overground part of the medicinal herb Goat's rue that is highly promising in this respect [1, 3, 4] is chosen as the subject of the study.
Goats' rue (*Galega officinalis* L.) is the herbaceous perennial plant of the leguminous family (*Fabaceae*) [5]. The overground portion of the plant is predominantly used for the treatment of diabetes mellitus. In the USA, UK, and Bulgaria the goat's rue herb is used in official medicine on the early stages of diabetes type II or as the part of its complex treatment [6].
Goat's rue herb contains alkaloids (0.1 - 0.2%), such as derivatives of guanidine – galegine and hydroxyl-4-galegine; derivatives of quinazoline – peganine and 2-3-oxy-quinazolone-4. Besides of alkaloids, the aboveground portion contains glycoside galuteolin, flavonoids (kaempferol, quercetin, rutin), tannins, hydroxycinnamic acids, saponins, bitters, pectins, essential amino acids, vitamins (carotene, ascorbic acid) and chromium salts [7-9].
The hypoglycemic effect of the goat's rue was investigated in 1927, however, the literature data concerning the sugar-lowering effects of the herb and seeds are controversial. Some researches consider that the hypoglycemic action is only found in the plant extracts that contain alkaloids which cause their toxicity. According to the literature data, guanidine alkaloids found in *Galega officinalis* induce prolonged hypoglycemic effect [5].

However, data occur that the alkaloid-free fracture of the plant extract also has a sugar-reducing effect. In fact, it has a less prolonged effect after discontinuation of the herbal drug application, but it is less toxic [5, 6, 8, 10]. The hypoglycemic effect of its non-alkaloid fracture can be explained by the presence of flavonoids, phytol [2, 11], ethylic ether of palmitinic acid, phytosterols (campesterol, stigmasterol), α-amyrin [1, 3, 12, 13] considering scientific data which were found in research literature.

Flavonoids and hydroxycinnamic acids exhibit a wide range of biological activity. In particular, researches confirm the properties of flavonoids to improve the glycosidic metabolism, to regulate lipid metabolism, hormonal and fermentative balance in the body, thus preventing the progress of diabetes and its complications [14-16]. Scientific publications demonstrate the hypoglycemic potency of rutin that acts as an antioxidant and antidiabetic agent [17].

The derivatives of guanidine present in *Galega officinalis*, as well as the chromium salts, provide for the raw material hypoglycemic properties, facilitate regulation of adrenal glands, and improve functions of the digestive system [7, 9].

Microelements of goat's rue also augment the hypoglycemic effect of the raw material [16]. In physiological concentration, copper potentiates the hypoglycemic effect of insulin, accelerates processes of glucose oxidation, suppresses a decomposition of glycogen and facilitates its accumulation in the liver. Manganese has a property to enhance the hypoglycemic effect of insulin; it increases a glycolytic activity and intensity of fat breakdown, reduces level of lipids in the body, and prevents a fatty degeneration of liver. Trace element selenium acts as a co-factor in the processes of stabilization of damaged cells, as well as prevents absorption of pro-oxidant microelements [16].

Amongst the objectives of our research were the following: to determine the microelement composition of goat's rue (*Galega officinalis* L.), collected in the Western part of Ukraine, to receive aqueous-alcoholic extracts of different concentration and to compare occurrence of components and quantities of phenolic compounds in the obtained ethanolic extracts from the plant above-ground parts.

Materials and Methods

The samples of dried goat's rue herb, collected and identified by authors in western regions of Ukraine during the flowering period (July-August 2016), were used in the experimental research.

Trace elements content in aboveground portions of *Galega officinalis* L. were quantitatively determined by atomic absorption spectrometry with electrothermal atomization (AAS / EA) with a Zeeman background correction after total microwave – assisted digestion of plant material samples with the further determination by the official method, in accordance with the European Pharmacopoeia [18], of standard additions, as described in our earlier publication [19].

To select an appropriate extractant, it is important to consider a chemical composition of the raw material, as well removing of the proper group of biologically active substances (BAS), and also economic efficiency and safety. The flavonoid glycosides are known to be highly soluble in water or in alcohol-water mixtures [20]. While obtaining extracts of BAS from goat's rue, the correlation of the raw material to the end product was 1:10. Based on the above mentioned item ethanol was used as the extractant in different concentrations from 20 to 96%. Extraction procedures were conducted at room temperatures. Extracts were obtained by the method of fractional maceration during the same time for all series, and then extracts were combined and filtered through the filter paper [21].

Phenolic compounds were identified by the method of high-performance liquid chromatography (HPLC) [22]. The liquid chromatograph Agilent 1200 with chromatographic column XTerra C18 (4.6x250 mm; 5 μm particle size) was used in order to conduct the research with the HPLC technique.

To prepare the mobile phase A we have used a solution of sodium dihydrogen phosphate monohydrate – 0.6 g/l, adjusted to pH value 2, 5 with the phosphoric acid; while for the mobile phase B – acetonitrile solution. The flow rate 1.0 ml/min at the gradient elution; injection volume of samples 100 μL; the column thermostat temperature 25 °C. Diode array detection was carried out at the wavelength 330 nm. Identification of the substances was conducted by comparison of retention time values of peaks on chromatogram of the tested solution with the corresponding values for standard substances (chlorogenic acid, caffeic acid, ferulic acid, rosmarinic acid, luteolin-7-glycoside, rutin, hyperoside, apigenin-7-glycoside, luteolin, quercitin, apigenin, kaempferol). All the reagents and preparation of the solutions complied with the requirements of the State Pharmacopoeia of Ukraine [23]. Typical chromatograms, obtained within the investigations, are shown on the figures 1, 2.

Quantitative determination of the total flavonoids in aboveground portion of goat's rue, expressed as rutin, was performed by the spectrophotometric method, applying aluminium complex formation in ethanolic medium with the further spectra measurements [24, 25]. Optical density and absorption spectra were measured on the spectrophotometer Carry-50 [23].

Test solution: Place 1,0 mL of the tested ethanolic extract in the 25 mL measuring flask, add 5 mL of aluminium chloride reagent R in ethanol R, and adjust the volume with 70% ethanol.

Compensation liquid. Dilute 1,0 mL of the test solution to 25.0 mL with the 70% ethanol.

Reference solution. 0.05 g (precisely weighed quantity) of standard rutin sample (Sigma) was diluted in 50 mL of 70% ethanol during warming on the water-bath, cooled and adjusted the volume of solution with the same solvent up to 100 mL. 5 mL of 5% aluminium chloride reagent R was added to 1 mL of the obtained solution and adjusted with the 70% ethanol in the 25 mL measuring flask.

*Compensation liquid.*1 mL of solution of the standard sample of rutin was placed in the 25 mL measuring flask and adjusted the volume with 70% ethanol.

Optical density of the obtained tested solutions and the reference solution was measured by the spectrophotometer at the wavelength of 410 nm in the cuvette with the 10 mm layer 30 min after preparation.

The contents of the total flavonoids (X) in the raw material, in per cent, expressed as rutin, were calculated according to the following formula:

$$X=\frac{Ax \cdot m0}{A0}$$

where: Ax – optical density of the tested solution;
A0 – optical density of the reference solution;
m0 – mass of rutin, in mg.

Results and Discussion

Identification of flavonoids and hydroxycinnamic acids by the HPLC method was conducted in goat's rue (*Galega officinalis* L.) herb extracts. The technique allowed determining a qualitative composition of the analyzed medicinal plant material. Totally the presence of 48 phenolic compounds was found. 7 substances were identified: caffeic acid, ferulic acid, chicoric acid, rutin, quercetin, hyperoside, and apigenin. Amongst the identified compounds rutin had the highest contents.

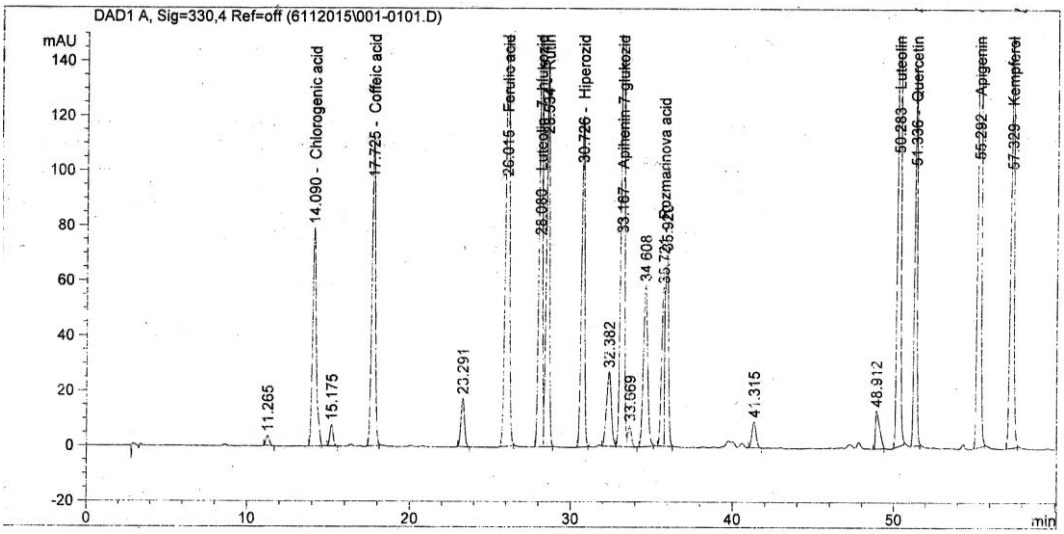

Fig 1: The typical HPLC chromatogram of standard solutions' mixture of flavonoids and hydroxycinnamic acids

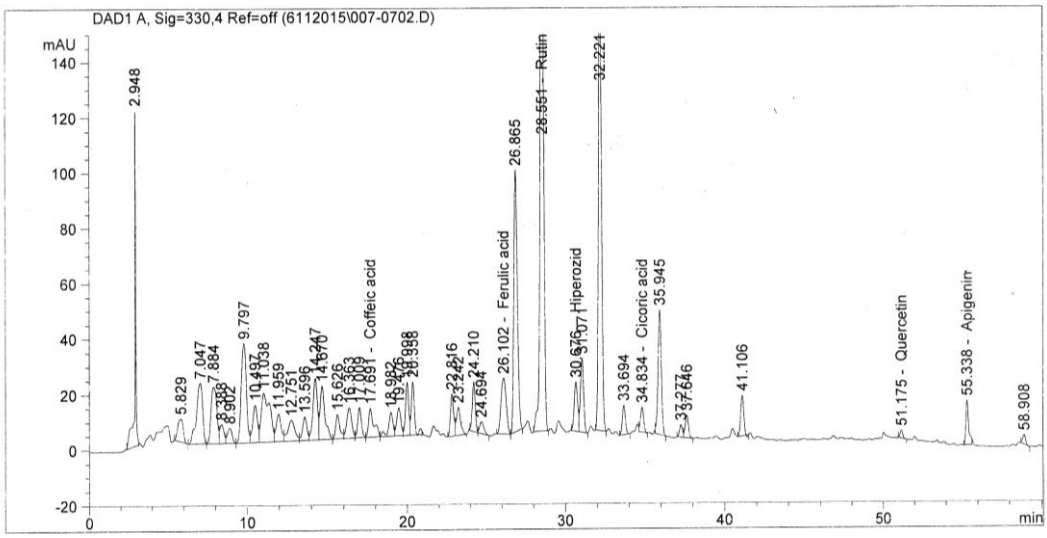

Fig 2: The typical HPLC chromatogram of 50% ethanolic extract of *Galega officinalis* L. herb, obtaining during detection of flavonoids and hydroxycinnamic acids

The effect of ethanol concentration on the degree of extraction of flavonoids was studied. It was based on determination of the yield of the total flavonoids by the method of differential spectrophotometry. The optimal range of values for the ethanol concentration for extraction of flavonoids was established as the result of the conducted research, it comprises 50-60%. The highest content of the total of flavonoids (0.0972%) was obtained applying 50% solution of ethanol. Therefore, for further research 50% alcoholic extract of the Goat's rue herb should be taken.

Results of quantitative measurement of the flavonoids content in the Goat's rue (*Galega officinalis* L.) herb extract, expressed as rutin, are illustrated in the Fig. 4.

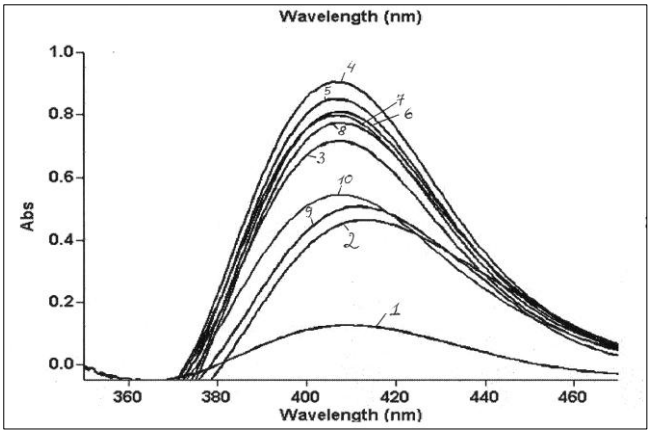

Fig 3: Differential electronic absorption spectra under conditions of quantitative measurement of flavonoids in the Goat's rue herb extracts of different concentration of ethanol: 1-20%; 2-30%; 3-40%; 4-50%; 5-60%;6-70%; 7-80%; 8-90%; 9-96% ethanol; 10- rutin

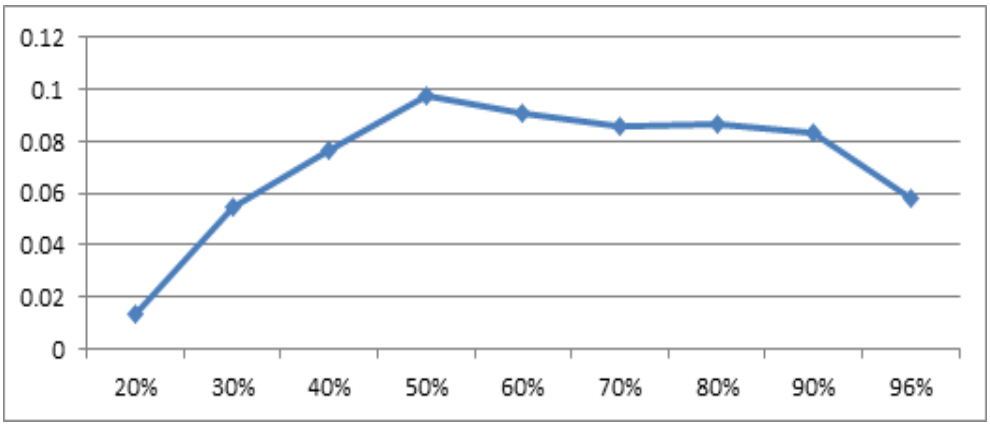

Fig 4: Diagram of dependence for total amount of flavonoids in obtained extracts of above-ground parts of *Galega officinalis* L. from ethanol concentration

As it is shown in the diagram (Fig.4), the highest content of flavonoids' totality was obtained using 50% ethanol as an extractant.

Trace elements are being studied as possible active principles in hypoglycemic plants. The mineral fraction of certain medicinal herbs from indigenous folk medicines exhibits higher glucose tolerance factor action than the organic fraction of the plants [26]. The high concentrations of K, Ca, Cr, Mn, Cu, and Zn in several anti-diabetic medicinal plants have been made responsible for stimulation of insulin action [27]. Deficiencies of copper can be an important factor in the development and evolution of chronic diseases such as cardiovascular dysfunction and diabetes [28]. Manganese deficiency is associated with growth impairment, bone abnormalities, diabetic-like carbohydrate changes, incoordination, and increased susceptibility to convulsions [29].

For a more complete estimation of aerial parts of Goat's rue, the study of the contents of microelements have been carried out in the current research by atomic absorption spectroscopy with electrothermal atomization (AAC/EA) on VARIAN AA 240Z after mineralization in the microwave Milestone Start D. The found quantities of trace elements in the analyzed samples were as follows: manganese – 23.8 µg/g, copper – 14.2 µg/g, selenium – 0.27 µg/g, lead – 0.13 µg/g, cadmium – 0.013 µg/g. The determined amounts of lead and cadmium in the investigated samples of above-ground parts of *Galega officinalis* L. comply with the requirements of the European Pharmacopoeia (Monograph 01/2012:1433 "Herbal Drugs) [18]: maximum 1.0 ppm of cadmium and maximum 5.0 ppm of lead, and therefore, might be considered as non-toxic natural source of the essential microelements manganese, copper and selenium.

Conclusion

The development of modern methods for standardization of the extracts from *Galega officinalis* L. and obtained on its basis the standardized drugs of appropriate quality, which allow predicting the expected hypoglycemic outcome, is highly relevant for the treatment of diabetes mellitus.

The results of detection of phenolic compounds in aqueous-alcoholic extracts from aerial portion of goat's rue showed occurrence of 48 compounds, amongst which 7 compounds were identified, such as caffeic acid, ferulic acid, chicoric acid, rutin, quercetin, hyperoside, and apigenin.

The optimal extractant for the maximum extraction of the flavonoids' totality from goat's rue (*Galega officinalis* L) herb has been determined and the fact that the highest yield of flavonoids (0.097%) was extracted with 50% alcoholic extract has been established.

Experimental data, obtained within determination of microelements of goat's rue (*Galega officinalis* L.) herb, confirmed the presence of manganese, copper and selenium, which are able to enhance the anti-diabetic effect of the medicinal plant material.

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